VOLUME 28 · NUMBER 6 · FEBRUARY 20 2010

JOURNAL OF CLINICAL ONCOLOGY

BIOLOGY OF NEOPLASIA

The PI3K Pathway As Drug Target in Human Cancer

Kevin D. Courtney, Ryan B. Corcoran, and Jeffrey A. Engelman

From the Department of Medicine, Harvard Medical School; Department of Medical Oncology, Dana-Farber Cancer Institute; Beth Israel Deaconess Medical Center Cancer Center; Massachusetts General Hospital Cancer Center, Boston, MA.

Submitted August 3, 2009; accepted November 18, 2009; published online ahead of print at www.jco.org on January 19, 2010.

Supported by an American Society of Clinical Oncology Young Investigator Award and a Genentech Dana-Farber/Harvard Cancer Center (DF/HCC) Kidney Cancer Career Development Award (K.D.C.), by National Institutes of Health K08 Grants No. CA120060, No. R01CA140594, and No. R01CA137008, by National Cancer Institute Lung Special Program of Research Excellence (SPORE) Grant No. P50CA090578, DF/HCC Gastrointestinal Cancer SPORE Grant No. P50 CA127003, the Ellison Foundation Scholar award (J.A.E.), and the American Association for Cancer Research Stand Up To Cancer program.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Corresponding author: Jeffrey A. Engelman, MD, PhD, Massachusetts General Hospital Cancer Center, 149 13th St, Charlestown, MA, 02129; e-mail: jengelman@partners.org.

0732-183X/10/2806-1075/$20.00

DOI: 10.1200/JCO.2009.25.3641

ABSTRACT

The phosphatidylinositol 3-kinase (PI3K) signaling axis impacts on cancer cell growth, survival, motility, and metabolism. This pathway is activated by several different mechanisms in cancers, including somatic mutation and amplification of genes encoding key components. In addition, PI3K signaling may serve integral functions for noncancerous cells in the tumor microenvironment. Consequently, therapeutics targeting the PI3K pathway are being developed at a rapid pace, and preclinical and early clinical studies are beginning to suggest specific strategies to effectively use them. However, the central role of PI3K signaling in a large array of diverse biologic processes raises concerns about its use in therapeutics and increases the need to develop sophisticated strategies for its use. In this review, we will discuss how PI3K signaling affects the growth and survival of tumor cells. From this vantage, we will consider how inhibitors of the PI3K signaling cascade, either alone or in combination with other therapeutics, can most effectively be used for the treatment of cancer.

J Clin Oncol 28:1075-1083. © 2010 by American Society of Clinical Oncology

INTRODUCTION

It has been more than 20 years since phosphatidylinositol 3-kinase (PI3K) was first discovered. The transforming ability of viral oncoproteins relied on an association with a PI3K lipid kinase activity.[1-4] Over the ensuing years, studies established the central role of PI3K signaling in several cellular processes critical for cancer progression, including metabolism, growth, survival, and motility. Inappropriate co-option of PI3K signaling is one of the most frequent occurrences in human cancer.[5,6] Consequently, significant efforts have been made to generate inhibitors of the PI3K pathway to treat cancers. However, it remains unknown which cancers will benefit most from these treatments and how to best use such therapeutics. In addition, the many possible untoward biologic sequelae of PI3K inhibition may limit the potential therapeutic gain of PI3K pathway inhibition. Here we will review data demonstrating the role of PI3K in tumor development and maintenance. We will compare the different potential therapeutic options for inhibiting this pathway and how their efficacy may be affected by the mechanism of PI3K pathway activation in a particular cancer. Finally, we will discuss the emerging data assessing the relative benefits of PI3K pathway inhibitors used as single agents versus combination therapies to treat cancer.

PI3K SIGNALING CASCADE REGULATES CELL GROWTH AND SURVIVAL

There are three classes of PI3Ks grouped according to structure and function. Class I_A PI3K is the one most clearly implicated in human cancer.[7] Class I_A PI3Ks consist of a regulatory subunit and a catalytic subunit. Three mammalian genes, *PIK3R1*, *PIK3R2*, and *PIK3R3*, encode p85α (p85α, p55α, and p50α isoforms), p85β, and p55γ regulatory subunits, respectively, which by convention are referred to collectively as p85.[5,7,8] The catalytic isoforms, p110α, p110β, and p110δ, are the products of three genes, *PIK3CA*, *PIK3CB*, and *PIK3CD*.[5,8] As will be discussed in greater detail below, both *PIK3CA* and *PIK3R1* are somatically mutated in cancers, and these mutations promote activation of the PI3K pathway.[9-12]

Class I_A PI3Ks are activated by growth factor stimulation through receptor tyrosine kinases (RTKs).[13-15] The regulatory subunit, p85, directly binds to phosphotyrosine residues on RTKs and/or adaptors.[16] This binding relieves the intermolecular inhibition of the p110 catalytic subunit by p85 and localizes PI3K to the plasma membrane where its substrate, phosphatidylinositol 4,5-bisphosphate ($PI[4,5]P_2$), resides.[15,16] PI3K can also be stimulated by activated Ras, which directly binds p110.[17] Additionally, the p110β catalytic subunit can be activated by G-protein coupled receptors.[8]

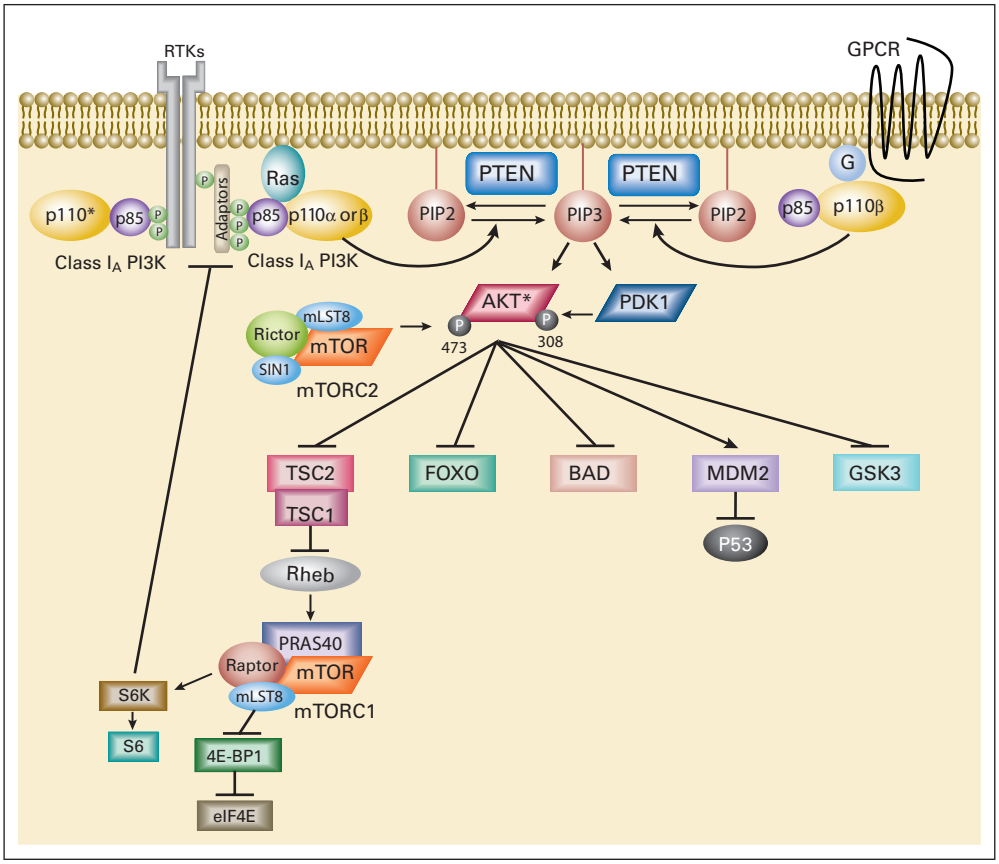

Fig 1. The phosphatidylinositol 3-kinase (PI3K) signaling cascade. PI3K signaling impacts on cell growth, survival, and metabolism. Arrows represent activation, while bars reflect inhibition. A negative feedback loop has been described from the downstream target S6 kinase (S6K) to the adaptor protein IRS-1. RTK, receptor tyrosine kinase; GPCR, G-protein coupled receptor; P, phosphate; G, G protein; PTEN, phosphatase and tensin homolog; IRS-1, insulin receptor substrate 1; eIF4E, eukaryotic initiation factor 4E; S6, ribosomal S6 protein; PIP_2, phosphatidylinositol 4,5-bisphosphate; mTORC2, rapamycin (mTOR) –containing protein complex 2. (*) p110 alpha, beta, or delta.

PI3K phosphorylates PIP_2 on the 3′OH position to produce $PI(3,4,5)P_3$ (PIP_3; Fig 1). The tumor suppressor phosphatase and tensin homolog deleted on chromosome 10 (PTEN) dephosphoryates PIP_3 to PIP_2, thereby terminating PI3K-dependent signaling. PIP_3 propagates intracellular signaling by directly binding pleckstrin homology (PH) domains of various signaling proteins.[18] $PI(3,4,5)P_3$ brings two PH domain-containing serine/threonine kinases, phosphoinositide-dependent kinase 1 (PDK1) and AKT, into close proximity. PDK1 activates AKT by phosphorylating AKT at threonine 308.[19-21] PI3K-AKT signaling promotes cell growth and survival by several mechanisms. AKT promotes cell survival by inhibiting proapoptotic Bcl-2 family members BAD and BAX.[5,18] AKT also impedes negative regulation of the transcription factor NF-κB, leading to increased transcription of antiapoptotic and prosurvival genes.[22] Phosphorylation of Mdm2 by AKT antagonizes p53-mediated apoptosis, and AKT negatively regulates forkhead transcription factors, thereby reducing production of cell death-promoting proteins.[22] AKT also phosphorylates TSC2, thereby inhibiting the rheb GTPase activity of the TSC1/TSC2 dimer. Activated rheb stimulates the mammalian target of rapamycin (mTOR) –containing protein complex mTORC1, leading to increased p70 S6 kinase activity.[5] Activation of mTORC1 results in increased protein synthesis by phosphorylation of eukaryotic initiation factor 4E and the ribosomal S6 protein.[5] While mTORC1 relays signals following PI3K-AKT activation, a second mTOR complex, mTORC2, contributes to complete AKT activation by phosphorylating AKT on serine 473.[23-25] Of note, activation of the mTORC1 target, S6 kinase, negatively feeds back to diminish PI3K activation. S6 kinase can phosphorylate and inhibit the adaptor protein insulin receptor substrate 1, thereby inhibiting insulin or insulin-like growth factor 1–mediated PI3K activation.[26-28]

Inhibitors of PI3K Signaling in Cancer Treatment

Inhibition of PI3K signaling can diminish cell proliferation, and in some circumstances, promote cell death. Consequently, components of this pathway present attractive targets for cancer therapeutics. A number of PI3K pathway inhibitors have been developed and are being evaluated in preclinical studies and in early clinical trials. Rapamycin analogs, such as temsirolimus and everolimus, that specifically inhibit mTORC1 are the most advanced in the clinic, and they have received US Food and Drug Administration approval for the treatment of advanced renal cell carcinoma.[29] The role for rapamycin analogs in the treatment of cancer has been extensively reviewed elsewhere and thus will not be discussed further.[30] In this review, we will discuss the potential therapeutic roles for other PI3K pathway inhibitors. These include PI3K inhibitors (both pan-PI3K and isoform-specific PI3K inhibitors), dual PI3K-mTOR inhibitors that are catalytic site inhibitors of the p110 isoforms and mTOR (the kinase component of both mTORC1 and mTORC2), mTOR catalytic site inhibitors, and AKT inhibitors. Not only do these agents have the capacity to inhibit cancer cell proliferation and survival signals as described above, but they may also impact tumor angiogenesis, metastasis, and metabolism. Due to space limitations, the impact of PI3K inhibition on tumor angiogenesis and cell motility is discussed in the Appendix (online only).

PI3K and Insulin Signaling: Potential Toxicity and Pharmacodynamic Marker of PI3K Inhibition

PI3K signaling has a central role in mediating the effects of insulin on cellular metabolism that is conserved throughout eurkaryotic evolution.[5] Noninsulin-dependent diabetes mellitus, marked by insulin insensitivity, is associated with diminished PI3K response to insulin signaling.[5,31] Several transgenic and knockout mice harboring alterations in p85, p110, PTEN, or AKT2 validate the functional significance of this pathway on glucose homeostasis.[31-34] These data suggest that insulin resistance may be observed in patients treated with PI3K pathway inhibitors, and indeed this may be used as a pharmacodynamic marker of target inhibition in patients. As will be discussed further below, initial phase I studies with PI3K pathway inhibitors have demonstrated some signs of insulin resistance, but this has not been a dose-limiting toxicity. While both p110α and p110β appear to play specific roles in insulin signaling, studies suggest that glucose homeostasis is predominantly mediated by p110α.[35,36] Inhibitors of p110α, but not p110β or p110δ, have been shown to inhibit insulin-stimulated glucose uptake in adipocytes and to block insulin-mediated glucose regulation in mice.[36] Consequently, in settings where p110β appears to be the critical PI3K catalytic isoform mediating transformation (eg, some PTEN-deficient tumors, see below), a p110β-specific inhibitor may offer efficacy with decreased risk of insulin resistance compared with a pan-PI3K inhibitor.

ACTIVATION OF PI3K SIGNALING IN CANCER

PI3K signaling is activated in human cancers via several different mechanisms.[6,11-13] Increased PI3K signaling is often due to direct mutational activation or amplification of genes encoding key components of the PI3K pathway such as *PIK3CA* and *AKT1*, or loss of *PTEN*.[6,9,12,37-41] Genetic alterations in several components of the PI3K signaling pathway have been reported and are summarized in Table 1. PI3K also can be activated by genetic mutation and/or amplification of upstream RTKs, and possibly by mutationally activated Ras.[7,17] The mechanism of PI3K activation in an individual cancer may suggest the most effective type of therapeutic to inhibit the pathway.

Somatic Alterations of PI3K Pathway Components in Cancer

The most common genetic alteration of the PI3K signaling pathway found in human cancer is inactivation of the *PTEN* tumor suppressor gene. Inactivation of *PTEN* leads to loss of its lipid phosphatase activity, causing accumulation of PIP_3.[56,57] The majority of somatic mutations in *PTEN* lead to protein truncation. However, missense mutations that typically abrogate PIP_3 phosphatase activity are also commonly noted.[58] While most *PTEN* mutations are sporadic, germline mutations in *PTEN* are noted in hereditary neoplastic disorders, such as Cowden disease.[59] Homozygous and hemizygous deletions of *PTEN* are also observed in human cancers.[38,45] Transcriptional repression and epigenetic silencing of *PTEN*, typically through promoter hypermethylation, is also a mechanism of *PTEN* inactivation.[42,43] Because there are both genetic and epigenetic causes for PTEN loss, accurate assessment of a cancer's PTEN status remains challenging and may ultimately require reliable measurements of protein expression.

More recently, somatic mutations in *PIK3CA* have been identified in a variety of human tumors, including breast, colon, and endometrial cancers and glioblastomas (see Catalogue of Somatic Mutations in Cancer, http://www.sanger.ac.uk/genetics/CGP/cosmic).[6,7,9,12,39] Most of these mutations cluster to two hot spot regions in exons 9 and 20.[6,12] Exon 20 encodes the catalytic domain of p110α, and mutations in this domain may constitutively activate its enzymatic activity. Exon 9 encodes the helical domain of p110α, and

Table 1. Genetic Alterations of the PI3K Signaling Pathway in Cancer

Gene	Alteration	Comments	Tumor Types	References
PTEN	LOF mutation	Truncation; loss of phosphatase activity	Bladder, brain, breast, cervical, colorectal, endometrial, gastric, head and neck, kidney	COSMIC, Li et al[38]
	Deletion	Homozygous or hemizygous	Leukemia, liver, lung, lymphoma, melanoma, ovary, prostate, thyroid	
	Epigenetic silencing	Transcriptional repression, usually by promoter hypermethylation	Breast, colon, melanoma	Garcia et al,[42] Goel et al,[43] Berns et al[44]
PIK3CA	GOF mutation	Exon 9 (E542K, E545K) helical domain; exon 20 (H1047R) catalytic domain	Breast, colorectal, glioblastoma, endometrial, cervical, esophageal, gastric, head and neck, liver, lung, lymphoma, ovarian, pancreatic, prostate, thyroid	COSMIC, Samuels et al,[6] Samuels et al[12]
	Amplification	Increased protein levels and activity	Breast, cervical, gastric, lung, ovarian, prostate	Sun et al,[45] Byun et al,[46] Campbell et al,[47] Ma et al,[48] Rácz et al[49]
PIK3R1	GOF mutation	Loss of C-terminal inhibitory domain; constitutive activity	Brain, colon, ovarian	Mizoguchi et al,[10] Philp et al[11]
AKT1	GOF mutation	Pleckstrin homology domain, membrane localization, and constitutive activation	Breast, colon, endometrial, melanoma, ovarian	Shoji et al,[40] Carpten et al,[50] Davies et al[51]
AKT2	GOF mutation	Kinase domain mutation	Colorectal	Parsons et al[52]
	Amplification		Breast, colon, lymphoma, pancreas	Cheng et al,[53] Cheng et al,[54] Bellacosa et al[55]
AKT3	GOF mutation	Pleckstrin homology domain, membrane localization, and constitutive activation	Melanoma	Davies et al[51]
PDK1	GOF mutation	Kinase domain mutation	Colorectal	Parsons et al[52]

Abbreviations: LOF, loss of function; COSMIC, Catalogue of Somatic Mutations in Cancer at www.sanger.ac.uk/genetics/CGP/cosmic; GOF, gain of function.

these mutations de-repress an inhibitory interaction between the N-terminal SH2 domain of p85 and the p110α catalytic subunit.[60,61] A smaller cluster of mutations is also found in the N-terminal p85 interacting domain. Interestingly, these mutations increase the lipid kinase activity of p110α but do not appear to alter the interaction between p110α and p85α.[9,12]

Expression of these *PIK3CA* mutants leads to increased oncogenic potential in vitro and in vivo.[9,37] They cause constitutive signaling along the PI3K pathway in the absence of growth factors and therefore seem to obviate the usual obligate interactions with tyrosine phosphorylated RTKs and/or adapters. Thus, it is intriguing that some studies have suggested that the presence of these mutations confers resistance to therapies targeting RTKs.[44,62] Expressing mutated *PIK3CA* in fibroblasts and mammary epithelial cells results in transformation, growth factor-independent proliferation, and resistance to apoptosis.[9,63,64] Additionally, transgenic mice with lung-specific induction of the kinase-domain mutant p110α H1047R develop lung adenocarcinomas.[65] In addition to these activating mutations, amplification of *PIK3CA* is also observed frequently in ovarian cancer and other tumors, but how amplification affects PI3K activation is less clear.[39]

Mutations in the p85 regulatory subunit *PIK3R1* are also observed in a variety of human cancers, including glioblastomas, ovarian cancers, and colorectal cancers.[10,11] Mutations in *PIK3R1* generally produce either truncations or in-frame deletions that often localize to the inter-SH2 domain of p85α. Structural analyses suggest that the iSH2 domain of p85 interacts with the C2 domain of p110.[60] Thus, it seems likely that these p85α mutations also activate PI3K signaling by relieving the inhibitory effect of p85 on p110.[11,66] Laboratory studies suggest that these mutations also lead to constitutive PI3K signaling.[11,66]

Mutations in AKT family genes encoding for AKT1, AKT2, and AKT3 have also been identified in human cancers. A single amino acid substitution, E17K, in the lipid-binding PH domain of AKT1 has been identified in breast, colorectal, endometrial, and ovarian cancers, as well as melanoma.[40,50,51] This amino acid change alters AKT1 lipid binding, presumably leading to constitutive membrane localization in the absence of PIP_3. However, although phosphorylation on Ser473 was constitutive in this mutant, T308 phosphorylation was still responsive to PI3K activation.[50] Thus, it is unclear if PI3K inhibitors will effectively decrease AKT signaling in cancers with these mutations. The E17K mutation has also been identified in AKT3 in some melanomas.[51] In addition, mutations affecting the kinase domain of AKT2 have been found in colorectal cancers; however, the functional consequences of these mutations have not been assessed.[52] Amplification of AKT2 has also been reported in human tumors.[53,54]

PI3K Activation by Receptor Tyrosine Kinases and Ras

In normal epithelial cells, PI3K is often activated downstream of RTK signaling. In cancers, these RTKs are often mutated, amplified, or overexpressed, causing aberrant PI3K activation. When therapies targeting RTKs are effective, they invariably lead to loss of PI3K signaling.[67] For example, PI3K is activated by epithelial growth factor receptor (EGFR) in lung cancers harboring somatic activating mutations in EGFR, and by human epidermal growth factor receptor 2 (HER2) in breast cancers with HER2 amplification.[65,68-70] In these cancers, EGFR or HER2 phosphorylates the kinase-dead ErbB3 that, in turn, directly binds and activates PI3K.[65,68-70] Thus, when these cancers are successfully treated with EGFR- and HER2-targeted therapies, respectively, PI3K signaling is turned off and the cells undergo cell death. Similarly, glioblastomas frequently exhibit PI3K activation, either through integration of signaling from multiple activated RTKs, such as the constitutively active EGFRvIII mutant, or through the combined activation of RTKs and loss of PTEN.[71,72]

The small GTPase Ras is also frequently mutated in human cancers, and PI3K is an effector of Ras-mediated oncogenic signaling.[73] Early studies showed that Ras directly bound p110, and provided a direct link between Ras and PI3K.[74] In addition, functional studies demonstrated that PI3K activation appears to be crucial for tumor initiation. For example, expression of a dominant-negative p85α lacking the p110-binding domain inhibited Ras-mediated transformation.[8,75] In addition, expression of a p110α mutant that does not directly bind Ras inhibited K-Ras–induced lung adenocarcinomas in genetically engineered mouse models.[76] Similarly, deletion of *Pik3r1* and *Pik3r2* abrogated *K-Ras* G12D-induced lung tumorigenesis.[65] Although PI3K activation may be necessary for K-Ras–induced tumorigenesis, preliminary studies suggest that inhibition of PI3K signaling alone may not be sufficient to shrink established tumors in vivo or effectively treat *K-Ras*–mutated cancer cell lines in vitro.[65,77] These findings underscore the difference between killing established cancers and blocking tumorigenesis and cell transformation. Furthermore, these studies suggest that established cancers with *KRAS* mutations may not be sensitive to single-agent PI3K pathway inhibitors.

Potential Roles for p110β, p110δ, and p110γ in Transformation

While activating mutations in *PIK3CA* are frequently identified in human cancers, no oncogenic mutations have been verified in p110β, p110δ, or the class I_B catalytic isoform p110γ. Although rare somatic single-residue substitutions have been found in p110β and p110γ (www.sanger.ac.uk/perl/genetics/CGP/cosmic), the function of these substitutions is unknown. Despite the lack of evidence for activating mutations in these other p110 catalytic isoforms, recent work has demonstrated the oncogenic potential of p110β, p110δ, and p110γ. Interestingly, unlike p110α, overexpression of wild-type p110β, p110δ, or p110γ is transforming in cell culture.[13] Although expression of the γ and δ isoforms is normally restricted to leukocytes, increased p110δ (as well as p110β) has been identified in some colon and bladder cancers, as well as in glioblastomas.[13] p110δ appears to provide the critical PI3K activity in acute myelogenous leukemia, while p110γ is upregulated by the *Bcr-Abl* oncogene in chronic myelogenous leukemia.[78,79]

Recent data also suggest a prominent role for p110β in PTEN-deficient tumors. Targeted deletion of *pten* in the mouse prostate results in prostatic intraepithelial neoplasia and carcinoma.[80] Concomitant ablation of p110β, but not p110α, decreased PI3K proliferation signaling and prevented prostate tumorigenesis.[35] Similarly, inducible depletion of p110β, but not p110α, using short hairpin RNA in PTEN-deficient human cancer cell lines extinguished PI3K-mediated signaling and inhibited growth in vitro and in vivo.[81] Deletion of p110β also abrogated transformation of mouse embryo fibroblasts by activated Ras or EGFR mutants to a more pronounced extent than did p110α loss.[35] These studies suggest that although cancers driven by *PIK3CA* mutations are candidates for treatment with p110α-specific inhibitors, treatment of PTEN-deficient cancers may require agents with activity against p110β.

PI3K PATHWAY INHIBITORS ENTERING THE CLINIC: PRECLINICAL AND EARLY CLINICAL DATA

A number of potential therapeutics targeting the PI3K signaling cascade have been generated. We will consider four different classes of PI3K pathway inhibitors: dual PI3K-mTOR inhibitors, PI3K inhibitors (that do not inhibit mTOR), AKT inhibitors, and mTOR catalytic site inhibitors. Table 2 summarizes PI3K pathway inhibitors in clinical trials.

Dual PI3K-mTOR Inhibitors

The catalytic domains of the p110 subunits and mTOR are structurally similar, because they all belong to the phosphatidylinositol kinase–related kinase family of kinases. Many chemical inhibitors under development inhibit both mTOR and the p110 catalytic subunits. These are termed dual PI3K-mTOR inhibitors. When compared with the other types of PI3K pathway inhibitors, dual PI3K-mTOR inhibitors have the possible advantage of inhibiting all PI3K catalytic isoforms, mTORC1, and mTORC2. Thus, they should effectively turn off this pathway completely and overcome feedback inhibition normally observed with mTORC1 inhibitors (ie, rapamycin analogs) that may limit their efficacy (Fig 1).[28] However, it remains unknown if dual PI3K-mTOR inhibitors will be tolerable at doses that effectively inhibit all p110 isoforms and mTOR, or if their use will necessitate sacrificing complete inhibition of one or more of the potential targets.

For many years, the PI3K inhibitor LY294002, a dual PI3K-mTOR inhibitor, has been extensively used in preclinical studies. Although LY294002 is unsuitable for patient use, the backbone structure of this compound has been exploited in the design of novel PI3K inhibitors.[7,90] SF-1126 (Semafore, Indianapolis, IN) is a prodrug of LY294002 that is conjugated to a tetra-peptide designed to target tumor vasculature, and this compound has demonstrated efficacy in solid tumor xenograft models.[7,90,91] In a phase I study of SF-1126, mTORC1 inhibition in cancers was demonstrated by decreased S6 phosphorylation.[82] No responses were observed, but stable disease was achieved in 11 of 28 patients below the maximum tolerated dose, without consistent effects on blood glucose.[82]

Other dual PI3K-mTOR inhibitors, such as NVP-BEZ235 and NVP-BGT226 (Novartis, Basel, Switzerland) and XL765 (Exelixis, San Francisco, CA) have entered phase I testing in clinical trials.[30,90,92] There have been several preclinical evaluations of NVP-BEZ235.

Table 2. PI3K Pathway Inhibitors in Clinical Trials

Targets	Compound (Company)	Study Population	Reported Efficacy/Responses	Reported Toxicities	References
PI3K/mTOR	SF-1126 (Semafore Pharmaceuticlas)	Phase I: advanced solid tumors	SD in 11 of 28 pts	DLT: grade 3 diarrhea (one pt)	Chiorean et al[82]
	NVP-BEZ235 (Novartis)	Phase I/II: advanced solid tumors (breast cancer-enriched)	N/A	N/A	ClinicalTrials.gov
	NVP-BGT226 (Novartis)	Phase I/II: advanced solid tumors (including breast cancer)	N/A	N/A	ClinicalTrials.gov
	XL765 (Exelixis)	Phase I: refractory solid tumors	SD in five of 36 pts	Most frequent (> 10%) AEs: elevated liver enzymes, nausea, diarrhea Other AEs: anorexia/ hypophosphatemia, rash, vomiting, and neurologic complaints	LoRusso et al[83]
PI3K	PX-866 (Oncothyreon)	Phase I: advanced solid tumors	SD in two of six pts (initial dosing cohorts)	Possible AEs: abdominal discomfort, mild diarrhea No DLTs in early dosing cohorts	Jimeno et al[84]
	XL147 (Exelixis)	Phase I: advanced solid tumors	SD (> 6 months) in six of 39 pts; one pt with HR CaP with normalized PSA	AEs: grade 3 rash (DLT in two of three pts at highest tested dose); grade 3 arterial thrombosis (one pt); grade 2 transaminitis (one pt); grade 1 hyperglycemia (four pts)	Shapiro et al[85]
	NVP-BKM120 (Novartis)	Phase I: solid tumors	N/A	N/A	Markman et al[86]
	GDC-0941 (Genentech/ Piramed)	Phase I: advanced solid tumors	Evidence for potential antitumor activity in three of 19 pts	Most frequent AEs: Grade 1 to 2 nausea, fatigue, diarrhea, dysgeusia, and peripheral edema	Wagner et al[87]
	CAL-101 (Calistoga Pharmaceuticals)	Phase I: relapsed/refractory CLL or B-cell NHLs	PR in two of six pts, SD in four of six pts in early dosing cohorts	No AEs grade > 1 in first two dosing cohorts	Flinn et al[88]
Akt (allosteric)	MK-2206 (Merck)	Phase I: advanced solid tumors	SD in six of 19 patients	Most frequent AEs: skin (47.1%) and GI (41.2%) toxicities; DLTs: grade 3 to 4 rash, grade 3 mucositis	Tolcher et al[89]

Abbreviations: SD, stable disease; DLT, dose-limiting toxicity; Pt, patient; N/A, results from patient treatment in clinical trials have not been reported to date; AE, adverse event; HR CaP, hormone-refractory prostate cancer; PSA, prostate-specific antigen; CLL, chronic lymphocytic leukemia; NHL, non-Hodgkin's lymphoma; PR, partial response.

NVP-BEZ235 slowed the growth of PTEN-deficient human cancer cell line xenografts in mice, and it was well tolerated with no significant changes in body weight.[92] Additionally, breast cancer cell lines with *HER2* amplification and/or *PIK3CA* mutations appeared to be particularly sensitive to these agents; however, it should be noted that only tumor stasis, and not tumor regression, was observed in vivo.[93] NVP-BEZ235 was further shown to induce apoptosis in estrogen-deprived estrogen receptor–positive breast cancer cells harboring either *PIK3CA* mutations or *PIK3CB* amplification.[94] In this study, RNA interference was also used to knock down p110α, p110β, or both in these cells. In some estrogen receptor–positive breast cancer cell lines harboring a *PIK3CA* mutation, dual knockdown of both p110α and p110β led to greater apoptosis following estrogen deprivation compared with knockdown of either isoform alone.[94] These results underscore a potential benefit of inhibiting both p110α and p110β, even in cancers that harbor specific genetic activation of one isoform.[94] A study with genetically engineered mice also demonstrated that NVP-BEZ235 was highly effective at shrinking murine lung tumors driven by a p110α H1047R transgene.[65] Recently, phase I results for the dual PI3K-mTOR inhibitor, XL765 were reported at the 45th American Society of Clinical Oncology (ASCO) annual meeting (2009). There were no responses, but stable disease was noted in five of 36 patients.[83] There was evidence of 50% to 80% pathway inhibition in surrogate tissue. However, it is unclear whether this level of inhibition will be sufficient to induce shrinkage in potentially responsive tumors, or whether more complete inhibition will be required. No significant changes in serum glucose were noted, although an augmentation in food-induced plasma insulin increases was observed.[83]

PI3K Inhibitors

The PI3K inhibitors can be divided into isoform-specific inhibitors or pan-PI3K inhibitors. Pan-PI3K inhibitors target all class I_A PI3K in the cancer. These include wortmannin derivatives such as PX-886 or wortmannin prodrugs such as the self-activating viridans modified by dextran linker moieties that are designed to increase permeability and extend serum half-life.[95-97] These agents exhibit cytostatic antitumor effects in vivo.[95-97] The presence of *PIK3CA* mutations appears to predict for sensitivity to PX-866 across an array of cancer cell lines derived from different tissues of origin.[77] Interestingly, PTEN loss also appears to predict for PX-866 sensitivity, despite its relatively low efficacy toward p110β.[77] Animals treated with PX-866 experienced hyperglycemia with decreased glucose tolerance as a major toxicity of PI3K inhibition, but this could be overcome with the oral antidiabetic agent pioglitozone.[98] Phase I clinical trial results for other pan-PI3K inhibitors have also been reported. Of 19 patients with solid tumors treated with GDC-0941 (Piramed/Genentech, Slough, United Kingdom/South San Francisco, CA), three demonstrated potential signs of antitumor activity.[87] Another pan-PI3K inhibitor, XL147, produced durable disease control in six of 39 treated patients. As observed with the dual PI3K-mTOR inhibitor XL765, plasma glucose levels were minimally affected by XL147, although an augmentation of food-induced plasma insulin increases was noted.[85]

A possible advantage of isoform-specific PI3K inhibitors is that they may be tolerated at doses resulting in more complete target inhibition with fewer adverse effects. Isoform-specific inhibitors that selectively inhibit p110α, β, δ, or γ catalytic subunits are under investigation in preclinical studies.[99,100] Indeed, a p110δ-specific inhibitor tested for refractory non-Hodgkin's lymphoma and chronic lymphocytic leukemia induced responses in 6 out of 12 patients (presented at the 45th Annual Meeting of ASCO in 2009).[80]

AKT Inhibitors

Both adenosine triphosphate (ATP) mimetics and noncatalytic-site AKT inhibitors are under active clinical development.[90,101] Cancers with *AKT1* mutations and *AKT1* and *AKT2* amplifications may be expected to be among the more sensitive to AKT inhibitors. However, this class of inhibitors will not block the non-AKT effectors of PI3K signaling and, paradoxically, could actually increase PI3K-dependent activation of those effectors via loss of negative feedbacks. This is especially important in light of the recent findings that the PDK1 substrate SGK3, and not AKT, may play a more prominent role in promoting PI3K-dependent viability in some cancers harboring *PIK3CA* mutations.[102] Despite these findings, a recent study demonstrated that a noncatalytic site AKT1/AKT2 inhibitor was effective against breast cancer cell lines with *PIK3CA* mutations and *HER2* amplifications.[101] Phase I results for the allosteric pan-AKT inhibitor MK-2206 (Merck, Whitehouse Station, NJ) showed stable disease in six of 19 patients and decreases in CA125 in patients with ovarian cancer. Adverse effects included rash and hyperglycemia.[89]

mTOR Catalytic Site Inhibitors

Rapamycin interacts with FKBP12 in mammalian cells to form a complex that directly binds to the FKBP12-rapamycin-binding domain of mTOR in mTORC1, but not in mTORC2.[103,104] Conversely, ATP-competitive mTOR inhibitors target the kinase domain of mTOR to impede the activity of both mTORC1 and mTORC2. Inhibiting mTORC2 would provide the theoretical advantage of blocking AKT activation. An ATP-competitive mTOR inhibitor might be more effective than rapamycin because, by blocking AKT activation, it would mitigate the activation of PI3K that often accompanies mTORC1 inhibition (ie, de-repression of the negative feedback, Fig 1). Intriguing preclinical data are emerging from studies of these compounds that shed new light on the potential limitations of rapamycin analogs. Feldman et al[105] demonstrated that two mTOR kinase domain inhibitors, PP242 and PP30, inhibit both mTORC1 and mTORC2. Unlike acute rapamycin treatment, which activates AKT, PP242 administration to mice suppressed AKT activation in tissues. PP242 was also a more effective inhibitor of proliferation than rapamycin.[105] Surprisingly, the improved efficacy of PP242 appeared to be due to more effective mTORC1 inhibition, rather than through its additional inhibition of mTORC2.[105] Similarly, the ATP-competitive mTOR inhibitor Torin1 impeded cell proliferation predominantly via its effects on mTORC1, not mTORC2.[106] Both PP242 and Torin1 were more effective inhibitors of 4E-BP1 phosphorylation and cap-dependent RNA translation than rapamycin.[103,105,106] Three additional ATP-competitive mTOR inhibitors—WAY-600, WYE-687, and WYE-354—have been shown to inhibit proliferation of a variety of cancer cell lines more effectively than rapamycin, causing G1 cell cycle arrest, and in some cases, apoptosis.[104]

Although the clinical results with PI3K pathway inhibitors are preliminary, their efficacy has modest at best. For their effective development, it will be imperative to understand why these drugs fail to produce a response when they do. Is the lack of activity due to inadequate inhibition of the target, or because complete inhibition of the target is not sufficient to produce antitumor activity? Indeed, most of the studies to date have not assessed this issue systematically. To

answer this question, future studies with quantitative pharmacodynamic assessments will be required to determine the degree of target inhibition. For example, a study with even a small number of patients with favorable genotypes (eg, *PIK3CA* mutants or *HER2* amplified) that correlates pharmacodynamic responses of PI3K pathway inhibition with outcomes may prove invaluable in identifying the reasons for lack of efficacy.

POTENTIAL CLINICAL USES FOR PI3K PATHWAY INHIBITORS

Thus far, preclinical studies have shown that PI3K pathway inhibitors may have significant single-agent activity in a few types of genetically defined cancers: HER2-amplified breast cancers, cancers with *PIK3CA* mutations, and PTEN-deficient cancers.[65,77,92,93,101] To this point, data suggest that cancers with KRAS mutations may be fairly resistant to PI3K pathway inhibitors.[65,77] Consequently, it seems likely that the presence of *KRAS* mutations will limit the efficacy of single-agent PI3K pathway inhibitors in cancers harboring both *KRAS* and *PIK3CA* mutations, such as many colon cancers.

In addition to these genetically defined settings, there may be other opportunities to target the PI3K pathway. For example, PI3K pathway inhibitors may be effective agents in the treatment of certain cancers that acquire resistance to RTK inhibitors. Cancers that are sensitive to receptor tyrosine kinase inhibitors (TKIs) have PI3K under the exclusive control of that RTK.[67] When a TKI works, it leads to downregulation of PI3K activity. For example in HER2-amplified breast cancers, trastuzumab disrupts the interaction between ErbB2 and ErbB3, resulting in ErbB3 dephosphorylation and loss of interaction with PI3K.[107] Furthermore, the presence of an activating *PIK3CA* mutation or depletion of *PTEN* correlates with a poor response to trastuzumab, presumably because these cancers fail to downregulate PI3K signaling in response to the anti-HER2 therapy. Furthermore, when cancers that were initially sensitive to TKIs subsequently develop resistance, they invariably find a way to maintain PI3K signaling in the presence of the TKI.[67] These cancers therefore may be susceptible to addition of a PI3K pathway inhibitor to the TKI to re-induce remissions. This is an attractive approach in HER2-amplified breast cancers, because they appear to be sensitive to PI3K pathway inhibitors even before they develop resistance to anti-HER2–based therapies. Indeed, the HER2-amplified breast cancer cell lines with *PIK3CA* mutations are resistant to trastuzumab, and this can be overcome with treatment with GDC-0941.[44,107] Similarly, PTEN loss, or activating mutations in *PIK3CA,* confers resistance to lapatinib, which can be overcome by treatment with NVP-BEZ235.[68]

Potential of Combining PI3K With MEK Pathway Inhibitors

When cancers are sensitive to TKIs (ie, oncogene-addicted to an RTK), the TKI usually leads to downregulation of PI3K and other pathways, including the MEK-MAPK pathway. Thus, it remains unclear whether single-agent PI3K pathway inhibitors will promote dramatic responses (comparable to gefitinib in *EGFR*-mutant lung cancers or imatinib in chronic myelogenous leukemia), even in sensitive cancers. Most models of cancers that are sensitive to single-agent PI3K pathway inhibitors have demonstrated tumor stasis in vivo rather than frank tumor regressions.[91-93,95-97] Consequently, it may be necessary to combine PI3K pathway inhibitors with other agents to induce dramatic responses. Furthermore, there may be cancers that show no response to single-agent PI3K pathway inhibitors that will respond to PI3K pathway inhibitors combined with other therapies. For example, inhibition of PI3K signaling with NVP-BEZ235 failed to shrink established *Kras* G12D-driven lung tumors.[65] However, combined PI3K and MAPK pathway inhibition by treatment with NVP-BEZ235 and the MEK inhibitor ARRY-142886 led to marked tumor regression in this *Kras* lung cancer model.[65] Similarly, combined PI3K and MEK inhibition was required to effectively shrink EGFR-mutant lung cancers in genetically engineered mouse models.[108] Findings such as these are spurring the biotechnology and pharmaceutical industries to combine therapeutic inhibitors of these two pathways.

SUMMARY

Great strides are being made in our understanding of the diverse roles that PI3K signaling plays in cancer initiation, progression, and maintenance. Novel therapeutics targeting different components of this pathway are demonstrating efficacy in an array of human cancer types in preclinical studies, and these drugs are being carried forward into clinical trials. There is growing preclinical evidence that some genetically defined cancer subtypes may be the most sensitive to single-agent PI3K pathway inhibitors. These include cancers with *PIK3CA* activating mutations, loss of PTEN, and breast cancers with HER2 amplification. However, it remains to be determined whether these sensitive cancers will demonstrate stable disease or tumor shrinkage in response to single-agent therapeutics. Conversely, cancers harboring activated Ras mutants appear to be insensitive to PI3K pathway inhibition alone. In such cases, effective treatment with PI3K inhibitors may require concomitant therapies targeting MAPK signaling.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: None **Consultant or Advisory Role:** Jeffrey A. Engelman, Novartis (C), AstraZeneca (C), Millennium Pharmaceuticals (C), Bristol-Myers Squibb (C), Hoffman-La Roche (C), OSI Pharmaceuticals (C), Schering-Plough (C), MedImmune (C), Daiichi Sankyo (C) **Stock Ownership:** None **Honoraria:** None **Research Funding:** Kevin D. Courtney, Genentech; Jeffrey A. Engelman, Novartis **Expert Testimony:** None **Other Remuneration:** None

AUTHOR CONTRIBUTIONS

Conception and design: Kevin D. Courtney, Ryan B. Corcoran, Jeffrey A. Engelman
Manuscript writing: Kevin D. Courtney, Ryan B. Corcoran, Jeffrey A. Engelman
Final approval of manuscript: Kevin D. Courtney, Ryan B. Corcoran, Jeffrey A. Engelman

REFERENCES

1. Jia S, Roberts TM, Zhao JJ: Should individual PI3 kinase isoforms be targeted in cancer? Curr Opin Cell Biol 21:199-208, 2009

2. Kaplan DR, Whitman M, Schaffhausen B, et al: Common elements in growth factor stimulation and oncogenic transformation: 85 kd phosphoprotein and phosphatidylinositol kinase activity. Cell 50:1021-1029, 1987

3. Sugimoto Y, Whitman M, Cantley LC, et al: Evidence that the Rous sarcoma virus transforming gene product phosphorylates phosphatidylinositol and diacylglycerol. Proc Natl Acad Sci U S A 81: 2117-2121, 1984

4. Whitman M, Kaplan DR, Schaffhausen B, et al: Association of phosphatidylinositol kinase activity with polyoma middle-T competent for transformation. Nature 315:239-242, 1985

5. Engelman JA, Luo J, Cantley LC: The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat Rev Genet 7:606-619, 2006

6. Samuels Y, Wang Z, Bardelli A, et al: High frequency of mutations of the PIK3CA gene in human cancers. Science 304:554, 2004

7. Yuan TL, Cantley LC: PI3K pathway alterations in cancer: Variations on a theme. Oncogene 27:5497-5510, 2008

8. Katso R, Okkenhaug K, Ahmadi K, et al: Cellular function of phosphoinositide 3-kinases: Implications for development, homeostasis, and cancer. Annu Rev Cell Dev Biol 17:615-675, 2001

9. Ikenoue T, Kanai F, Hikiba Y, et al: Functional analysis of PIK3CA gene mutations in human colorectal cancer. Cancer Res 65:4562-4567, 2005

10. Mizoguchi M, Nutt CL, Mohapatra G, et al: Genetic alterations of phosphoinositide 3-kinase subunit genes in human glioblastomas. Brain Pathol 14:372-377, 2004

11. Philp AJ, Campbell IG, Leet C, et al: The phosphatidylinositol 3′-kinase p85alpha gene is an oncogene in human ovarian and colon tumors. Cancer Res 61:7426-7429, 2001

12. Samuels Y, Velculescu VE: Oncogenic mutations of PIK3CA in human cancers. Cell Cycle 3:1221-1224, 2004

13. Kang S, Denley A, Vanhaesebroeck B, et al: Oncogenic transformation induced by the p110beta, -gamma, and -delta isoforms of class I phosphoinositide 3-kinase. Proc Natl Acad Sci U S A 103:1289-1294, 2006

14. Skolnik EY, Margolis B, Mohammadi M, et al: Cloning of PI3 kinase-associated p85 utilizing a novel method for expression/cloning of target proteins for receptor tyrosine kinases. Cell 65:83-90, 1991

15. Zhao L, Vogt PK: Class I PI3K in oncogenic cellular transformation. Oncogene 27:5486-5496, 2008

16. Carpenter CL, Auger KR, Chanudhuri M, et al: Phosphoinositide 3-kinase is activated by phosphopeptides that bind to the SH2 domains of the 85-kDa subunit. J Biol Chem 268:9478-9483, 1993

17. Shaw RJ, Cantley LC: Ras, PI(3)K and mTOR signalling controls tumour cell growth. Nature 441: 424-430, 2006

18. Cantley LC: The phosphoinositide 3-kinase pathway. Science 296:1655-1657, 2002

19. Alessi DR, James SR, Downes CP, et al: Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Balpha. Curr Biol 7:261-269, 1997

20. Currie RA, Walker KS, Gray A, et al: Role of phosphatidylinositol 3,4,5-trisphosphate in regulating the activity and localization of 3-phosphoinositide-dependent protein kinase-1. Biochem J 337:575-583, 1999

21. Majumder PK, Sellers WR: Akt-regulated pathways in prostate cancer. Oncogene 24:7465-7474, 2005

22. Duronio V: The life of a cell: Apoptosis regulation by the PI3K/PKB pathway. Biochem J 415: 333-344, 2008

23. Hresko RC, Mueckler M: mTOR.RICTOR is the Ser473 kinase for Akt/protein kinase B in 3T3-L1 adipocytes. J Biol Chem 280:40406-40416, 2005

24. Sarbassov DD, Ali SM, Sengupta S, et al: Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. Mol Cell 22:159-168, 2006

25. Sarbassov DD, Guertin DA, Ali SM, et al: Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 307:1098-1101, 2005

26. Carracedo A, Pandolfi PP: The PTEN-PI3K pathway: Of feedbacks and cross-talks. Oncogene 27:5527-5541, 2008

27. Harrington LS, Findlay GM, Gray A, et al: The TSC1-2 tumor suppressor controls insulin-PI3K signaling via regulation of IRS proteins. J Cell Biol 166:213-223, 2004

28. O'Reilly KE, Rojo F, She QB, et al: mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. Cancer Res 66:1500-1508, 2006

29. Ma WW, Adjei AA: Novel agents on the horizon for cancer therapy. CA Cancer J Clin 59:111-137, 2009

30. Meric-Bernstam F, Gonzalez-Angulo AM: Targeting the mTOR signaling network for cancer therapy. J Clin Oncol 27:2278-2287, 2009

31. Luo J, Sobkiw CL, Hirshman MF, et al: Loss of class IA PI3K signaling in muscle leads to impaired muscle growth, insulin response, and hyperlipidemia. Cell Metab 3:355-366, 2006

32. Cho H, Mu J, Kim JK, et al: Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta). Science 292:1728-1731, 2001

33. Taniguchi CM, Tran TT, Kondo T, et al: Phosphoinositide 3-kinase regulatory subunit p85alpha suppresses insulin action via positive regulation of PTEN. Proc Natl Acad Sci U S A 103:12093-12097, 2006

34. Ueki K, Yballe CM, Brachmann SM, et al: Increased insulin sensitivity in mice lacking p85beta subunit of phosphoinositide 3-kinase. Proc Natl Acad Sci U S A 99:419-424, 2002

35. Jia S, Liu Z, Zhang S, et al: Essential roles of PI(3)K-p110beta in cell growth, metabolism and tumorigenesis. Nature 454:776-779, 2008

36. Knight ZA, Gonzalez B, Feldman ME, et al: A pharmacological map of the PI3-K family defines a role for p110alpha in insulin signaling. Cell 125:733-747, 2006

37. Bader AG, Kang S, Vogt PK: Cancer-specific mutations in PIK3CA are oncogenic in vivo. Proc Natl Acad Sci U S A 103:1475-1479, 2006

38. Li J, Yen C, Liaw D, et al: PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 275:1943-1947, 1997

39. Shayesteh L, Lu Y, Kuo WL, et al: PIK3CA is implicated as an oncogene in ovarian cancer. Nat Genet 21:99-102, 1999

40. Shoji K, Oda K, Nakagawa S, et al: The oncogenic mutation in the pleckstrin homology domain of AKT1 in endometrial carcinomas. Br J Cancer 101:145-148, 2009

41. Steck PA, Pershouse MA, Jasser SA, et al: Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat Genet 15:356-362, 1997

42. García JM, Silva J, Peña C, et al: Promoter methylation of the PTEN gene is a common molecular change in breast cancer. Genes Chromosomes Cancer 41:117-124, 2004

43. Goel A, Arnold CN, Niedzwiecki D, et al: Frequent inactivation of PTEN by promoter hypermethylation in microsatellite instability-high sporadic colorectal cancers. Cancer Res 64:3014-3021, 2004

44. Berns K, Horlings HM, Hennessy BT, et al: A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. Cancer Cell 12:395-402, 2007

45. Sun X, Huang J, Homma T, et al: Genetic alterations in the PI3K pathway in prostate cancer. Anticancer Res 29:1739-1743, 2009

46. Byun DS, Cho K, Ryu BK, et al: Frequent monoallelic deletion of PTEN and its reciprocal association with PIK3CA amplification in gastric carcinoma. Int J Cancer 104:318-327, 2003

47. Campbell IG, Russell SE, Choong DY, et al: Mutation of the PIK3CA gene in ovarian and breast cancer. Cancer Res 64:7678-7681, 2004

48. Ma YY, Wei SJ, Lin YC, et al: PIK3CA as an oncogene in cervical cancer. Oncogene 19:2739-2744, 2000

49. Rácz A, Brass N, Heckel D, et al: Expression analysis of genes at 3q26–q27 involved in frequent amplification in squamous cell lung carcinoma. Eur J Cancer 35:641-646, 1999

50. Carpten JD, Faber AL, Horn C, et al: A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. Nature 448:439-444, 2007

51. Davies MA, Stemke-Hale K, Tellez C, et al: A novel AKT3 mutation in melanoma tumours and cell lines. Br J Cancer 99:1265-1268, 2008

52. Parsons DW, Wang TL, Samuels Y, et al: Colorectal cancer: Mutations in a signalling pathway. Nature 436:792, 2005

53. Cheng JQ, Godwin AK, Bellacosa A, et al: AKT2, a putative oncogene encoding a member of a subfamily of protein-serine/threonine kinases, is amplified in human ovarian carcinomas. Proc Natl Acad Sci U S A 89:9267-9271, 1992

54. Cheng JQ, Ruggeri B, Klein WM, et al: Amplification of AKT2 in human pancreatic cells and inhibition of AKT2 expression and tumorigenicity by antisense RNA. Proc Natl Acad Sci U S A 93:3636-3641, 1996

55. Bellacosa A, de Feo D, Godwin AK, et al: Molecular alterations of the AKT2 oncogene in ovarian and breast carcinomas. Int J Cancer 64:280-285, 1995

56. Haas-Kogan D, Shalev N, Wong M, et al: Protein kinase B (PKB/Akt) activity is elevated in glioblastoma cells due to mutation of the tumor suppressor PTEN/MMAC. Curr Biol 8:1195-1198, 1998

57. Myers MP, Pass I, Batty IH, et al: The lipid phosphatase activity of PTEN is critical for its tumor suppressor function. Proc Natl Acad Sci U S A 95:13513-13518, 1998

58. Han SY, Kato H, Kato S, et al: Functional evaluation of PTEN missense mutations using in vitro phosphoinositide phosphatase assay. Cancer Res 60:3147-3151, 2000

59. Liaw D, Marsh DJ, Li J, et al: Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. Nat Genet 16:64-67, 1997

60. Huang CH, Mandelker D, Schmidt-Kittler O, et al: The structure of a human p110alpha/p85alpha complex elucidates the effects of oncogenic PI3Kalpha mutations. Science 318:1744-1748, 2007

61. Miled N, Yan Y, Hon WC, et al: Mechanism of two classes of cancer mutations in the phosphoinositide 3-kinase catalytic subunit. Science 317: 239-242, 2007

62. Engelman JA, Mukohara T, Zejnullahu K, et al: Allelic dilution obscures detection of a biologically significant resistance mutation in EGFR-amplified lung cancer. J Clin Invest 116:2695-2706, 2006

63. Isakoff SJ, Engelman JA, Irie HY, et al: Breast cancer-associated PIK3CA mutations are oncogenic in mammary epithelial cells. Cancer Res 65:10992-11000, 2005

64. Zhao JJ, Liu Z, Wang L, et al: The oncogenic properties of mutant p110alpha and p110beta phosphatidylinositol 3-kinases in human mammary epithelial cells. Proc Natl Acad Sci U S A 102:18443-18448, 2005

65. Engelman JA, Chen L, Tan X, et al: Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. Nat Med 14:1351-1356, 2008

66. Shekar SC, Wu H, Fu Z, et al: Mechanism of constitutive phosphoinositide 3-kinase activation by oncogenic mutants of the p85 regulatory subunit. J Biol Chem 280:27850-27855, 2005

67. Engelman JA, Settleman J: Acquired resistance to tyrosine kinase inhibitors during cancer therapy. Curr Opin Genet Dev 18:73-79, 2008

68. Eichhorn PJ, Gili M, Scaltriti M, et al: Phosphatidylinositol 3-kinase hyperactivation results in lapatinib resistance that is reversed by the mTOR/phosphatidylinositol 3-kinase inhibitor NVP-BEZ235. Cancer Res 68:9221-9230, 2008

69. Holbro T, Beerli RR, Maurer F, et al: The ErbB2/ErbB3 heterodimer functions as an oncogenic unit: ErbB2 requires ErbB3 to drive breast tumor cell proliferation. Proc Natl Acad Sci U S A 100:8933-8938, 2003

70. Moasser MM, Basso A, Averbuch SD, et al: The tyrosine kinase inhibitor ZD1839 ("Iressa") inhibits HER2-driven signaling and suppresses the growth of HER2-overexpressing tumor cells. Cancer Res 61:7184-7188, 2001

71. Mellinghoff IK, Wang MY, Vivanco I, et al: Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. N Engl J Med 353:2012-2024, 2005

72. Stommel JM, Kimmelman AC, Ying H, et al: Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies. Science 318:287-290, 2007

73. Lim KH, Counter CM: Reduction in the requirement of oncogenic Ras signaling to activation of PI3K/AKT pathway during tumor maintenance. Cancer Cell 8:381-392, 2005

74. Pacold ME, Suire S, Perisic O, et al: Crystal structure and functional analysis of Ras binding to its effector phosphoinositide 3-kinase gamma. Cell 103:931-943, 2000

75. Rodriguez-Viciana P, Warne PH, Khwaja A, et al: Role of phosphoinositide 3-OH kinase in cell transformation and control of the actin cytoskeleton by Ras. Cell 89:457-467, 1997

76. Gupta S, Ramjaun AR, Haiko P, et al: Binding of ras to phosphoinositide 3-kinase p110alpha is required for ras-driven tumorigenesis in mice. Cell 129: 957-968, 2007

77. Ihle NT, Lemos R Jr, Wipf P, et al: Mutations in the phosphatidylinositol-3-kinase pathway predict for antitumor activity of the inhibitor PX-866 whereas oncogenic Ras is a dominant predictor for resistance. Cancer Res 69:143-150, 2009

78. Hickey FB, Cotter TG: BCR-ABL regulates phosphatidylinositol 3-kinase-p110gamma transcription and activation and is required for proliferation and drug resistance. J Biol Chem 281:2441-2450, 2006

79. Sujobert P, Bardet V, Cornillet-Lefebvre P, et al: Essential role for the p110delta isoform in phosphoinositide 3-kinase activation and cell proliferation in acute myeloid leukemia. Blood 106:1063-1066, 2005

80. Salmena L, Carracedo A, Pandolfi PP: Tenets of PTEN tumor suppression. Cell 133:403-414, 2008

81. Wee S, Wiederschain D, Maira SM, et al: PTEN-deficient cancers depend on PIK3CB. Proc Natl Acad Sci U S A 105:13057-13062, 2008

82. Chiorean EG, Mahadevan D, Harris WB, et al: Phase I evaluation of SF1126, a vascular targeted PI3K inhibitor, administered twice weekly IV in patients with refractory solid tumors. J Clin Oncol 27:122s, 2009 (suppl; abstr 2558)

83. LoRusso P, Markman B, Tabernero J, et al: A phase I dose-escalation study of the safety, pharmacokinetics (PK), and pharmacodynamics of XL765, a PI3K/TORC1/TORC2 inhibitor administered orally to patients (pts) with advanced solid tumors. J Clin Oncol 27:146s, 2009 (suppl; abstr 3502)

84. Jimeno A, Hong DS, Hecker S, et al: Phase I trial of PX-866, a novel phosphoinositide-3-kinase (PI-3K) inhibitor. J Clin Oncol 27:156s, 2009 (suppl; abstr 3542)

85. Shapiro G, Kwak E, Baselga J, et al: Phase I dose-escalation study of XL147, a PI3K inhibitor administered orally to patients with solid tumors. J Clin Oncol 27:146s, 2009 (suppl; abstr 3500)

86. Markman B, Atzori F, Pérez-Garcia J, et al: Status of PI3K inhibition and biomarker development in cancer therapeutics. Ann Oncol doi: 10.1093/annonc/mdp347 [epub ahead of print on August 27, 2009]

87. Wagner AJ, Von Hoff DH, LoRusso PM, et al: A first-in-human phase I study to evaluate the pan-PI3K inhibitor GDC-0941 administered QD or BID in patients with advanced solid tumors. J Clin Oncol 27:146s, 2009 (suppl; abstr 3501)

88. Flinn IW, Byrd JC, Furman RR, et al: Preliminary evidence of clinical activity in a phase I study of CAL-101, a selective inhibitor of the p1108 isoform of phosphatidylinositol 3-kinase (PI3K), in patients with select hematologic malignancies. J Clin Oncol 27:156s, 2009 (suppl; abstr 3543)

89. Tolcher AW, Yap TA, Fearen I, et al: A phase I study of MK-2206, an oral potent allosteric Akt inhibitor (Akti), in patients (pts) with advanced solid tumor (ST). J Clin Oncol 27:146s, 2009 (suppl; abstr 3503)

90. Garcia-Echeverria C, Sellers WR: Drug discovery approaches targeting the PI3K/Akt pathway in cancer. Oncogene 27:5511-5526, 2008

91. Garlich JR, De P, Dey N, et al: A vascular targeted pan phosphoinositide 3-kinase inhibitor prodrug, SF1126, with antitumor and antiangiogenic activity. Cancer Res 68:206-215, 2008

92. Maira SM, Stauffer F, Brueggen J, et al: Identification and characterization of NVP-BEZ235, a new orally available dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor with potent in vivo antitumor activity. Mol Cancer Ther 7:1851-1863, 2008

93. Serra V, Markman B, Scaltriti M, et al: NVP-BEZ235, a dual PI3K/mTOR inhibitor, prevents PI3K signaling and inhibits the growth of cancer cells with activating PI3K mutations. Cancer Res 68:8022-8030, 2008

94. Crowder RJ, Phommaly C, Tao Y, et al: PIK3CA and PIK3CB inhibition produce synthetic lethality when combined with estrogen deprivation in estrogen receptor-positive breast cancer. Cancer Res 69:3955-3962, 2009

95. Blois J, Yuan H, Smith A, et al: Slow self-activation enhances the potency of viridin prodrugs. J Med Chem 51:4699-4707, 2008

96. Howes AL, Chiang GG, Lang ES, et al: The phosphatidylinositol 3-kinase inhibitor, PX-866, is a potent inhibitor of cancer cell motility and growth in three-dimensional cultures. Mol Cancer Ther 6:2505-2514, 2007

97. Smith A, Blois J, Yuan H, et al: The antiproliferative cytostatic effects of a self-activating viridin prodrug. Mol Cancer Ther 8:1666-1675, 2009

98. Ihle NT, Paine-Murrieta G, Berggren MI, et al: The phosphatidylinositol-3-kinase inhibitor PX-866 overcomes resistance to the epidermal growth factor receptor inhibitor gefitinib in A-549 human non-small cell lung cancer xenografts. Mol Cancer Ther 4:1349-1357, 2005

99. Chen JS, Zhou LJ, Entin-Meer M, et al: Characterization of structurally distinct, isoform-selective phosphoinositide 3′-kinase inhibitors in combination with radiation in the treatment of glioblastoma. Mol Cancer Ther 7:841-850, 2008

100. Torbett NE, Luna-Moran A, Knight ZA, et al: A chemical screen in diverse breast cancer cell lines reveals genetic enhancers and suppressors of sensitivity to PI3K isoform-selective inhibition. Biochem J 415:97-110, 2008

101. She QB, Chandarlapaty S, Ye Q, et al: Breast tumor cells with PI3K mutation or HER2 amplification are selectively addicted to Akt signaling. PLoS One 3:e3065, 2008

102. Vasudevan KM, Barbie DA, Davies MA, et al: AKT-independent signaling downstream of oncogenic PIK3CA mutations in human cancer. Cancer Cell 16:21-32, 2009

103. Guertin DA, Sabatini DM: The pharmacology of mTOR inhibition. Sci Signal 2:pe24, 2009

104. Yu K, Toral-Barza L, Shi C, et al: Biochemical, cellular, and in vivo activity of novel ATP-competitive and selective inhibitors of the mammalian target of rapamycin. Cancer Res 69:6232-6240, 2009

105. Feldman ME, Apsel B, Uotila A, et al: Active-site inhibitors of mTOR target rapamycin-resistant outputs of mTORC1 and mTORC2. PLoS Biol 7:e38, 2009

106. Thoreen CC, Kang SA, Chang JW, et al: An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1. J Biol Chem 284:8023-8032, 2009

107. Junttila TT, Akita RW, Parsons K, et al: Ligand-independent HER2/HER3/PI3K complex is disrupted by trastuzumab and is effectively inhibited by the PI3K inhibitor GDC-0941. Cancer Cell 15:429-440, 2009

108. Faber AC, Li D, Song Y, et al: Differential induction of apoptosis in HER2 and EGFR addicted cancers following PI3K inhibition. Proc Natl Acad Sci U S A 106:19503-19508, 2009

